APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 2003, p. 6577–6586 Vol. 69, No. 11
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.11.6577–6586.2003

Microaerophilic Cooperation of Reductive and Oxidative Pathways Allows Maximal Photosynthetic Membrane Biosynthesis in *Rhodospirillum rubrum*

Hartmut Grammel,[1]* Ernst-Dieter Gilles,[1] and Robin Ghosh[2]

Max Planck Institute for Dynamics of Complex Technical Systems, D-39106 Magdeburg,[1] and Department of Bioenergetics, Institute for Biology, University of Stuttgart, D-70550 Stuttgart,[2] Germany

Received 7 April 2003/Accepted 15 August 2003

The purple nonsulfur bacterium *Rhodospirillum rubrum* has been employed to study physiological adaptation to limiting oxygen tensions (microaerophilic conditions). *R. rubrum* produces maximal levels of photosynthetic membranes when grown with both succinate and fructose as carbon sources under microaerophilic conditions in comparison to the level (only about 20% of the maximum) seen in the absence of fructose. Employing a unique partial O_2 pressure (pO_2) control strategy to reliably adjust the oxygen tension to values below 0.5%, we have used bioreactor cultures to investigate the metabolic rationale for this effect. A metabolic profile of the central carbon metabolism of these cultures was obtained by determination of key enzyme activities under microaerophilic as well as aerobic and anaerobic phototrophic conditions. Under aerobic conditions succinate and fructose were consumed simultaneously, whereas oxygen-limiting conditions provoked the preferential breakdown of fructose. Fructose was utilized via the Embden-Meyerhof-Parnas pathway. High levels of pyrophosphate-dependent phosphofructokinase activity were found to be specific for oxygen-limited cultures. No glucose-6-phosphate dehydrogenase activity was detected under any conditions. We demonstrate that NADPH is supplied mainly by the pyridine-nucleotide transhydrogenase under oxygen-limiting conditions. The tricarboxylic acid cycle enzymes are present at significant levels during microaerophilic growth, albeit at lower levels than those seen under fully aerobic growth conditions. Levels of the reductive tricarboxylic acid cycle marker enzyme fumarate reductase were also high under microaerophilic conditions. We propose a model by which the primary "switching" of oxidative and reductive metabolism is performed at the level of the tricarboxylic acid cycle and suggest how this might affect redox signaling and gene expression in *R. rubrum*.

The growth of microorganisms is often studied under conditions considered aerobic (i.e., conditions under which availability of molecular oxygen is not a limiting factor) or anaerobic (i.e., conditions under which O_2 is excluded). The availability of O_2 thus provides a primary environmental signal for switching between growth modes, allowing the organism to utilize C and N sources by different metabolic strategies. For *Escherichia coli*, a fundamental difference in the metabolic pathways present during chemoheterotrophic growth under aerobic or anaerobic conditions is the presence or absence of the tricarboxylic acid (TCA) cycle enzymes α-ketoglutarate dehydrogenase (αKGDH) and succinate dehydrogenase (SDH) (33). Thus, under anaerobic conditions neither enzyme is present and the enzyme fumarate reductase (FRD) is expressed, allowing the TCA cycle to operate both reductively (from oxaloacetate to succinyl-coenzyme A [CoA]) and oxidatively (to α-ketoglutarate). In recent years it has been recognized that microaerophilic growth (i.e., under conditions in which O_2 levels are a limiting factor) is a distinct growth mode that is distinguishable at both the genetic and enzymatic levels from exclusive aerobic or anaerobic metabolism (35) and that is regulated by a complex interplay between different global regulators (21). However, a severe difficulty hampering the understanding of the factors regulating microaerophilic differential gene expression in general is that, often, few phenotypic markers are present.

Phototrophic bacteria may present an interesting system for studying microaerophilic metabolism, as many phenotypic markers, together with the now well-documented genetic accessibility, are available for both genetic and online analysis. In this study we focused on the photosynthetic bacterium *Rhodospirillum rubrum*, which was used in an experimental system for the study of the regulation and phenomenology of microaerophilic growth (and, by implication, its regulation by redox-mediated events) that is so far unique, due to the fact that growth media for this bacterium are available which allow maximal expression of phenotypic markers of anaerobic growth (such as levels of bacteriochlorophyll [BChl] and intracytoplasmic photosynthetic membranes [PM]) under microaerophilic conditions in darkness (19). By contrast, for other well-examined bacteria such as *Rhodobacter sphaeroides* or *Rhodobacter capsulatus* no comparable media have been developed so far. In addition, the genomic sequence of *R. rubrum* has been determined recently (http://genome.ornl.gov/microbial/), thus allowing a new level of genetic accessibility for this organism.

For *R. rubrum* the following experimental observations for different growth modes which are pertinent to the present study have been made. (i) *R. rubrum* grown aerobically (i.e., with nonlimiting O_2 levels) using succinate or malate as a carbon source (half-life [$t_{1/2}$], approximately 4 to 5 h) shows

* Corresponding author. Mailing address: Max Planck Institute for Dynamics of Complex Technical Systems, Sandtorstr. 1, D-39106 Magdeburg, Germany. Phone: 49-391-6110-255. Fax: 49-391-6110-527. E-mail: grammel@mpi-magdeburg.mpg.de.

only negligible amounts of PM (as indicated by the A_{880}/A_{660} ratio) (19), and the pH of the external medium rises steeply, reaching final values of about 11.0 in the stationary phase. The pH rise is succinate dependent and is probably caused by utilization of a succinate:H^+ symporter comparable to that seen with the Dct systems found in *E. coli* and *R. capsulatus* under aerobic conditions (18). We have annotated a homologous *dctA* gene in the published genomic sequence of *R. rubrum* (see Results). It is worth noting here that when Na succinate is replaced by NH_4 succinate, the pH rise is reduced due to the simultaneous uptake of NH_4 and the synthesis of PM is not affected (19). (ii) Anaerobic photoheterotrophic growth ($t_{1/2}$, approximately 20 h) with the well-known medium Sistrom A (32), which employs succinate (20 mM) as a carbon source and electron donor, leads to the maximal expression of BChl and PM at low levels of light intensity. Although low concentrations of glutamate and aspartate (0.6 and 0.3 mM, respectively) are present in Sistrom medium, their omission does not lead to a change in the cellular level of PM (R. Ghosh, unpublished data). The pH of the external medium remains stable at pH 6.8, which may indicate that under anaerobic conditions succinate uptake is not mediated by H^+ symport.

(iii) During fermentation of fructose or pyruvate in the dark, growth is slow ($t_{1/2} > 24$ h) and the pH of the medium drops sharply within several days due to the formation of acidic fermentation products.

(iv) In the dark in a microaerophilic (partial O_2 pressure [pO_2], below 0.5%) growth mode ($t_{1/2}$, approximately 7 to 10 h) with succinate as a carbon source, photosynthetic genes are only partially expressed. The yield of BChl-photosynthetic components is about 20% of that observed in anaerobic cultures grown in batch cultures under low levels of light intensity, with the pH rising eventually to pH 11.0 (19). However, as shown by Ghosh et al. (19), the low levels of BChl and PM are not a pH effect, as correction of the pH change either by increasing the buffer capacity or by online compensation has only a minimal effect upon BChl-PM expression.

(v) Microaerophilic growth in the presence of both fructose and succinate (M2SF medium) yields BChl-PM levels which are comparable to those observed with phototrophic growth under conditions of low levels of light intensity and is also associated with an only minimal increase in the pH of the external medium (19).

In this study, we set the stage for our ultimate goal, which is the mathematical modeling of the metabolism of *R. rubrum* under microaerophilic, as well as aerobic, photosynthetic, or fermentative conditions with the purpose of quantifying redox signaling at a level fundamentally different from those reported previously. As a prerequisite, we have performed a preliminary qualitative analysis of key enzymes in the microaerophilic growth mode, as defined by physiological and phenotypic markers observed under these conditions, and have identified similarities to and differences from a number of studies reported previously by other groups. Many of these previous studies were performed under almost exclusive conditions of photosynthetic, aerobic, or fermentative metabolism. We have also attempted to reformulate the central questions pertaining to redox signaling or signaling of the formation of the PM during microaerophilic growth in the context of a conceptual framework which also integrates different metabolic growth mode aspects of *R. rubrum*.

MATERIALS AND METHODS

Growth conditions. *R. rubrum* strain S1 (ATCC 11170) was used throughout this study. To obtain anaerobic (phototrophic) cultures, 200-ml screw-cap Pyrex flasks filled with M medium (32), which contains succinate as the main carbon source, were inoculated with cells (1%) grown aerobically in the dark. After incubation in the dark for 24 h to deplete oxygen from the medium, the cultures were illuminated with approximately 15 microeinsteins $m^{-2}\,s^{-1}$ and stirred with a magnetic stirrer at 260 rpm and 30°C. Irradiance was measured using a light meter (L-250; Li-Cor) equipped with a mini-quanta sensor (Walz, Effeltrich, Germany).

Cultivation in the bioreactor. Batch fermentations in M2SF medium (with succinate and fructose as carbon sources) were carried out in the dark in stainless steel 7-liter bioreactors (Biostat C; B. Braun Biotech, Melsungen, Germany) with a 4-liter working volume. A total of 200 ml (5%) of an anaerobic preculture grown in light was used as an inoculum. Fermentation parameters were controlled with a Simatic PCS7 automation system (Siemens, Munich, Germany). The temperature was held constant at 30°C, and the agitation rate was 100 rpm. The pH was measured with a glass electrode (Mettler-Toledo, Langenfeld, Germany). Due to the buffering capacity of the medium, no pH control was employed. The dissolved oxygen (DO) was monitored using a built-in membrane-type amperometric oxygen probe (InPro 6000; Mettler-Toledo). Polarization of the probe was carried out overnight, and calibration was performed by saturating the fermentation medium with sterile N_2 (0% pO_2) and sterile air (100% pO_2). The DO content was set by varying the airflow by the action of a combined mass flow meter-flow control valve.

To adjust microaerophilic growth conditions in the range of less than 0.5% pO_2, a control strategy which used the dependency of the pH on the DO concentration in the medium (DO-pH control; see Results) was employed. The process controller used two control loops for pH and pO_2, with the airflow functioning as the manipulated variable for both loops. The upper and lower limits of the oxygen input in the fermentor were fixed by predefined threshold values for pO_2 (0.2%) and pH (pH 6.8), respectively. Airflow was increased when the pH fell below the pH threshold value and decreased when the pO_2 reading exceeded the pO_2 threshold. As a result of this strategy the oxygen concentration in the fermentor was maintained at a constant level not obtainable by regulating the pO_2 with standard procedures.

Analytical procedures. Levels of cell density (measured according to A_{660} values) and formation of PMs (measured according to A_{880} values) were determined using a 1-cm-path-length cuvette with a Jasco V-560 spectrophotometer at 660 nm and 880 nm, respectively. Quantification of BChl was performed after extraction with acetone-methanol (7:2) at $\varepsilon_{770} = 75\ mM^{-1}\,cm^{-1}$ (11). The PM level was estimated using the A_{880}/A_{660} ratio as described previously (19). An A_{880}/A_{660} ratio of approximately 1.2 is characteristic of the highest PM levels obtained in anaerobic phototrophic cells grown at low levels of light intensity and is considered maximal (19).

Levels of *c*-type cytochromes were estimated (using the extinction coefficient for *c*-type hemes [$\varepsilon_{550\text{-}540} = 19.1\ mM^{-1}\,cm^{-1}$]) (10) by recording reduced (dithionite) minus oxidized [$K_3Fe(CN)_6$] difference spectra.

Concentrations of acetate, formate, fructose, and succinate in culture supernatants were determined by using appropriate enzyme kits (R-Biopharm, Darmstadt, Germany). After extensive extraction of lyophilized cells with chloroform, poly-β-hydroxybutyrate (PHB) was assayed spectrophotometrically as crotonic acid according to the method of Law and Slepecky (27). The protein content of cell extracts (see below) was measured with a bicinchoninic assay kit (Pierce, Rockford, Ill.), with bovine serum albumin as the standard. For the detection of 2-keto-3-deoxy-6-phosphogluconate, a method based on thin-layer chromatography was used (25). 6-Phosphogluconate levels were assayed after extraction of cells with formic acid (for 60 min at 4°C) and ion-chromatographic separation on a DX 600 high-pressure liquid chromatography system (Dionex) equipped with a Dionex ED50 electrochemical detector. Separation was carried out for 70 min using a Dionex AminoPac PA10 column with a linear gradient ranging from 100% eluent A (250 mM NaOH) to 100% eluent B (250 mM NaOH–1 M sodium acetate).

Preparation of cell extracts. Cells were harvested by centrifugation, washed, and resuspended in 50 mM potassium phosphate buffer (pH 7.2) or an appropriate Tris-HCl buffer. Aliquots (2 ml) were disrupted by sonification (with a Bandelin Sonopuls sonicator equipped with a standard sonication probe) on ice; four 30-s cycles at 20% of maximum power were used. Following cell breakage, extracts were centrifuged at 30,000 × *g* for 10 min to remove unbroken cells and

debris. The resulting crude extracts contained 0.5 to 5 mg of protein per ml. Crude extracts (designated microaerophilic) were obtained from DO-pH-controlled cultures (grown in the dark) with an A_{880}/A_{660} ratio of >1; aerobic cells were obtained mainly for pO_2-controlled cultures (grown in the dark) with an A_{880}/A_{660} ratio of 0.5 to 0.7. Anaerobic extracts were obtained from phototrophically grown cells.

Enzyme assays. Levels of ATP-dependent 6-phosphofructokinase (ATP-PFK) and PP_i-dependent 6-phosphofructokinase (PP_i-PFK) activity were determined by coupling the formation of fructose-1,6-bisphosphate to the oxidation of NADH via aldolase, triose phosphate isomerase, and glycerophosphate dehydrogenase. The assay mixture contained 500 mM imidazole-HCl (pH 7.2), $MgCl_2$ (3 mM), NADH (1.2 mM), ATP (or PP_i) (2 mM), fructose-6-phosphate (0.3 mM), rotenone (20 μM), aldolase (0.9 U), glycerophosphate dehydrogenase (3.1 U), and triose phosphate isomerase (9 U). The reaction was started by the addition of fructose-6-phosphate and monitored at 366 nm. For the determination of 1-PFK levels, fructose-1-phosphate was used to start the reaction. Glucose-6-phosphate dehydrogenase (G6PDH) was assayed as previously described (9). 2-Keto-3-deoxy-6-phosphogluconate aldolase (KDPG-aldolase) and 6-phosphogluconate dehydratase levels were assayed by measuring 6-phosphogluconate-dependent pyruvate formation in the presence of lactate dehydrogenase and NADH.

For the determination of ribulose-1,5-bisphosphate carboxylase (Rubisco) levels, the formation of 3-phosphoglycerate was coupled to the oxidation of NADH (monitored at 366 nm) with 3-phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase in the following reaction mixture: Tris-HCl (pH 8.2) (40 mM), $MgCl_2$ (10 mM), glutathione (10 mM), $NaHCO_3$ (50 mM), ATP (10 mM), NADH (400 μM), ribulose-1,5-bisphosphate (20 mM), 3-phosphoglycerate kinase (1.63 U), and glyeraldehyde-3-phosphate dehydrogenase (20.9 U). The reaction was started by the addition of ribulose-1,5-bisphosphate.

Levels of NADP-dependent isocitrate dehydrogenase (ICDH) activity were determined as previously described (9). Levels of the αKGDH complex were measured according to the levels of α-ketoglutarate-dependent reduction of NAD (8). For the determination of levels of the pyruvate dehydrogenase (PDH) complex, the same assay procedure (with sodium pyruvate as electron donor) was used. Levels of pyruvate-ferredoxin oxidoreductase were assayed in anaerobic cuvettes according to the levels of pyruvate-dependent reduction of methyl viologen. Prior to measurement, the assay and all required solutions were gassed with N_2. Measurement of the SDH complex was performed by oxidation of succinate by phenazine methosulfate in the presence of dichlorophenolindophenol, as described previously (1). FRD was assayed by fumarate-dependent oxidation of reduced flavin mononucleotide under anoxic conditions (22). For determination of levels of transhydrogenase (TH) activity, the reduction of NADP was measured by monitoring the reduction of thio-NADP with NADH at 415 nm in an assay mixture containing Tris-HCl (pH 7.6) (0.1 M), $MgCl_2$ (5 mM), ATP (5 mM), thio-NADP (250 μM), and NADH (200 μM). The extinction coefficient (ε_{415}) of thio-NADPH was determined to be 2.15 mM^{-1} cm^{-1}. Levels of NADP-dependent malic enzyme were determined as described previously (9).

Gene annotations relevant to the present study. When necessary, gene annotations of the *R. rubrum* DNA sequence data were performed using BLAST (http://www.ncbi.nlm.nih.gov) and NNPREDICT software. The latter is included in Vector NTI Suite version 5.5 (Informax) for DNA and protein analysis.

Nucleotide sequence accession number. The genes encoding pyruvate-formate lyase (*pfl*), acetate kinase (*act*), and phosphotransacetylase (*pta*) are present in the deposited genome sequence (http://genome.ornl.gov/microbial/) but have also been independently sequenced and annotated by us (EMBL gene bank accession no. AY150801). The aerobic succinate transporter *dctPQM* (18) was also annotated from the deposited genome sequence (http://genome.ornl.gov/microbial/). All attempts to annotate a coding sequence for G6PDH failed. However, we cannot exclude the possibility that this sequence is present in one of the gaps in the deposited gene sequence.

RESULTS

Batch cultivation of *R. rubrum* under microaerophilic conditions. To date, most studies of the metabolism of phototrophic organisms have dealt with either fully aerobic or anaerobic conditions due to the technical problems that arise when cells are investigated under very low levels of oxygen tension. A practical definition of the microaerophilic growth condition is critical for enzymatic analysis. It is well known that using these sensors, the measurement error with conventional oxygen probes below 5% pO_2 increases dramatically, making the determination and control of pO_2 at <1% (necessary for the microaerophilic state of *R. rubrum*) almost impossible to obtain. Preliminary studies with *R. rubrum* and direct control from an oxygen electrode confirmed this expectation (see below). In the event, growth could be observed but the kinetics were irreproducible and in all experiments high-level expression of the PM was unattainable.

We therefore chose a unique procedure for measurement and regulation of the microaerophilic state. Ghosh et al. had previously hypothesized (19) that the consumption of fructose occurs primarily by the well-known fermentative pathways and leads to a pH decrease, whereas the consumption of succinate occurs primarily by oxidative metabolism and leads to an increase in the medium pH due to the production of NaOH after the uptake of succinic acid by a succinate:H^+ symporter. In the microaerophilic state, these two processes are balanced by the availability of oxygen; the change in the medium pH is minimal until the stationary phase is reached. Under these conditions the production of phototrophic membranes in the dark, as measured by the near-IR absorption maximum at 880 nm, is optimal and equivalent to that observed under phototrophic reduced-light conditions (19). We consider this condition the functional definition of the microaerophilic state of *R. rubrum* as sensed by the cells. The strong and obligatory coupling of DO and pH in the microaerophilic state has a simple consequence: the control of the pO_2 at less than 1% should be achievable by a feedback loop which monitors the pH of the medium.

Thus, (i) when the pH rises, the organism is supplied with too much O_2 for maintenance of the microaerophilic state, causing the process control to decrease the air supply; (i) when the pH falls, too little O_2 is being supplied and growth is predominantly fermentative. In this case, the air supply is increased until the pH once again rises above the predefined pH threshold.

The induction of PM synthesis under these conditions is a clear marker for microaerophilic growth.

Growth kinetics under microaerophilic conditions. The time course of a typical microaerophilic batch cultivation of *R. rubrum* is shown in Fig. 1A. During the first stage of the cultivation the DO concentration was fixed at 5% air saturation by the use of a conventional proportional-integrational-differential controller. Remarkably, this relatively small pO_2 value was sufficient to support fully aerobic growth with respect to the oxygen-sensitive formation of PMs.

Thus, after inoculation of the fermentor with an anaerobic phototrophically grown inoculum the biosynthesis of PM was arrested. The dilution of cells containing high amounts of PM with those growing aerobically led to a rapid decrease (from 1.2 to 0.7) in the A_{880}/A_{660} ratio during the first 17 h. After 17 h, sufficient cell mass was present to decrease the pO_2 to below 1%, whereupon the DO-pH control loop was initiated to maintain the microaerophilic state. At this point, the biosynthesis of PM was induced and the A_{880}/A_{660} ratio rose. This kinetic behavior is identical to that observed in small culture flasks (19). The pO_2 probe reading did not exceed 0.3% (with the exception of rapid fluctuations) during the entire microaerophilic growth phase.

During the initial aerobic-growth phase, PHB accumulated

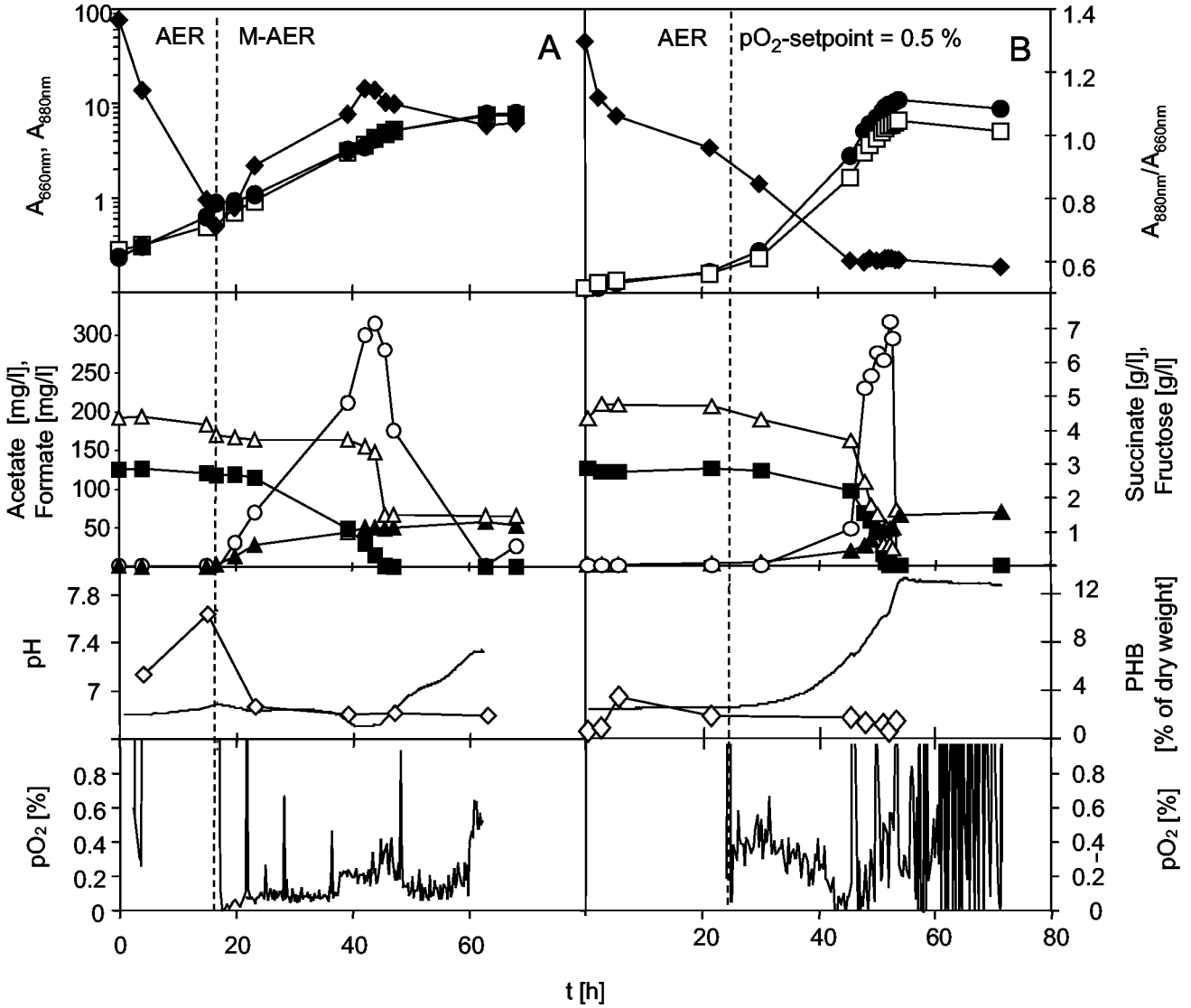

FIG. 1. Batch cultivation (using DO-pH control loop regulation [A] and pO_2 regulation [B]) of *R. rubrum* in M2SF medium. The onset of both control modes is indicated by dotted lines. AER, aerobic growth phase; M-AER, microaerophilic growth phase. Symbols: ●, $A_{660\ nm}$; □, $A_{880\ nm}$; ♦, $A_{880\ nm}/A_{660\ nm}$; ○, acetate; ▲, formate; ■, fructose; △, succinate; ◇, PHB. pH levels are indicated by lines without symbols. l, liter.

to more than 10% of the dry weight. Following the shift to microaerophilic growth after 17 h, the intracellular levels of PHB decreased to approximately 2% of the dry weight and remained constant for the rest of the fermentation.

Initially we expected a typical diauxic growth behavior, i.e., that fructose would only be consumed after microaerophilic growth conditions had been achieved by the aerobic oxidation of succinate. This proved not to be the case. In the event, both succinate and fructose were consumed simultaneously for the first 20 h, whereupon fructose was preferentially consumed to completion after 40 h whereas succinate was found in the culture supernatants throughout the cultivation. The preferential consumption of fructose in the microaerophilic state was correlated with the production of acetate (to a maximum value of 314 mg/liter [5.3 mM]) and, to a lesser extent, formate (maximal value, 70 mg/liter [1.5 mM]) in the growth medium. Following the complete depletion of fructose, the formation of formate ceased and acetate was rapidly depleted from the growth medium; the pH of the medium then rose correspondingly. The growth with succinate and acetate in this later phase was accompanied by a decrease in the A_{880}/A_{660} ratio; i.e., the production of PM was arrested.

The efficacy of our new control strategy for maintaining the microaerophilic state became clear when the same experiment was performed by conventional means, i.e., by utilizing solely an oxygen electrode for measurement and control (Fig. 1B). In this experiment, when the pO_2 value was regulated with a set point of 0.5 the loss of BChl (PM) in the cultures showed a monotonic decrease to extremely low values ($A_{880}/A_{660} = 0.58$ compared to $A_{880}/A_{660} = 1.0$ to 1.2 observed for the DO-pH control loop) after 50 h, which corresponded to the peak in acetate production observed for the DO-pH-controlled cultures (Fig. 1A). The A_{880}/A_{660} value of 0.58 was almost exclusively due to turbidity effects. The level of BChl under these

conditions was 0.46 nmol/mg of dry weight. The kinetics of acetate and formate production, however, were significantly different from those observed for the DO-pH-controlled cultures, showing significant and steep increases only after 30 h and not the slower monotonic rise after 17 h (corresponding to the production of PM) observed for the latter cultures. The uptake kinetics observed for succinate and fructose appeared initially to be similar to those observed for DO-pH-controlled cultures, but after 40 h (corresponding to the utilization of acetate from the culture medium) an interesting difference was observed. Whereas fructose was completely depleted from the medium in both DO-pH- and pO_2-controlled cultures, the latter cultures also showed the simultaneous depletion of succinate (Fig. 1B). This was also consistent with the much steeper rise in pH levels observed after 40 h for the pO_2-controlled cultures. Thus, under microaerophilic growth conditions in M2SF medium the preferential consumption of fructose in the DO-pH-controlled culture was accompanied by high levels of expression of PM, whereas the simultaneous consumption of fructose and succinate was accompanied by a lack of PM synthesis.

Finally, the initial growth levels of the DO-pH- and pO_2-controlled cultures showed an interesting difference. In contrast to the results seen with the DO-pH-controlled culture, the PHB increased only minimally (to 4% of the dry weight) during the initial growth phase, peaking at 5.3 h and then decreasing monotonically to a final constant level of 2%. We note here that in contrast to the DO-pH-controlled cultures, in which the initial pO_2 was maintained at 5% prior to the 17-h shift, the pO_2 in the pO_2-regulated cultures decreased rapidly in the initial phase so that at 5.3 h the level had dropped from 100 to 40%. This value decreased continuously so that at 24 h it had reached 0.5%, whereupon this value was maintained by the pO_2 control loop.

Key enzyme activities characteristic of the microaerophilic growth state. It is well known for both *E. coli* (33) and phototrophs (3) that a shift from aerobic to anaerobic metabolism is accompanied by the differential expression of specific marker enzymes. The results shown in Fig. 1 indicate that in the microaerophilic state *R. rubrum* retained elements from both aerobic and anaerobic metabolism, which appear to have been operating simultaneously. To understand the molecular basis for this metabolic coexistence, we have measured activities of a number of key enzymes in crude extracts (a table of the numerical values can be obtained at http://www.mpi-magdeburg.mpg.de/en/research/projects/1010/1014/1020/enzact.html) to determine which pathways were operating.

(i) Catabolism of fructose. The presence of fructose as a cosubstrate appears to be critical for maintaining the microaerophilic state. Thus, we addressed the following routes used for fructose degradation in *R. rubrum* by determination of the marker enzymes involved in fructose catabolism: the Embden-Meyerhof-Parnas (EMP) pathway, with key enzyme 6-PFK; the Entner-Doudoroff pathway, with key enzymes KDPG-aldolase and G6PDH; and the reductive pentose phosphate pathway (PPP), with key enzyme ribulose-1,5-bisphosphate carboxylase (Rubisco) or the oxidative PPP.

G6PDH, KDPG-aldolase, and 6-phosphogluconate dehydratase activities were not detected under any of the growth conditions studied. The validity of the assay was confirmed by a control experiment employing a glucose-grown exponential-phase culture of *E. coli* for which G6PDH activity was determined to be 29 nmol/min/mg of protein. In addition, 6-phosphogluconate was detected in *E. coli* extracts and the respective peak was absent in extracts of *R. rubrum* (data not shown). These data indicate that the oxidative PPP (and therefore the Entner-Doudoroff pathway) was not operating in *R. rubrum* regardless of the growth conditions used. On the other hand, the EMP marker enzyme 6-PFK, as well as an inorganic PP_i-PFK which has been described previously (30), was easily detected (Fig. 2). PP_i is provided by the membrane-bound proton-pump PP_i synthase (4, 5). Interestingly, the levels of PP_i-dependent activity were consistently two- to fivefold higher than those of ATP-PFK. In extracts of microaerophilic cells the activity of PP_i-PFK was considerably higher (97 nmol/min/mg of protein) than that seen with aerobically grown (38 nmol/mg of protein) or phototrophically grown (37 nmol/min/mg of protein) cells (Fig. 2). Production of PP_i-PFK was strongly induced by the presence of fructose in the culture medium and was most pronounced under microaerophilic conditions (97 nmol/mg of protein with succinate-fructose [M2SF medium]-grown cells versus 11 nmol/min/mg of protein with cells grown without fructose [M medium]). As fructose uptake can be accomplished by means of an inducible phosphotransferase system (15), leading to the formation of fructose-1-phosphate inside the cell, 1-PFK was also tested. In cell extracts obtained from phototrophic cultures grown in M medium, only marginal 1-PFK activities (presumably due to the absence of the inducer fructose) were observed. In summary, the results suggest that the breakdown of fructose occurs mainly through the EMP pathway.

(ii) NADPH-generating activities. The absence of G6PDH activity in *R. rubrum* under all growth conditions studied raises the question of how this organism synthesizes NADPH for biosynthetic processes. Although G6PDH is believed to be the main source for the regeneration of NADPH from NADP in most organisms, this role can also be fulfilled by the NADP-dependent ICDH and malic enzyme. ICDH and malic enzyme have been assayed in photoheterotrophically grown *R. rubrum* strains previously, with only ICDH showing significant cellular activity (23, 24). We found that ICDH levels were highest under aerobic conditions (381 nmol/min/mg of protein) followed by microaerophilic (220 nmol/min/mg of protein) and anaerobic phototrophic (182 nmol/min/mg of protein) conditions (Fig. 3). By contrast, L-malic enzyme (6.4 nmol/min/mg of protein) was detected under fully aerobic conditions; the activity was reduced sixfold under microaerophilic conditions and was undetectable under photosynthetic anaerobic conditions. We also determined the level of activity of the NADH-NADPH TH, which catalyses the reversible reduction of NADP by NADH coupled to a proton translocation across the cytoplasmic membrane (14). The ATP-stimulated NADP-reducing activity was highest in phototrophically grown cells and lowest in aerobically grown cells (5% of the phototrophic activity). However, in the microaerophilic state defined here, activity was observed at 30% of the activity level of phototrophically grown cells (Fig. 3). These activity levels were measured in the presence of 6 mM ATP, which stimulated the reaction (presumably due to the generation of proton motive force by the ATP synthase). The levels of BChl found in aer-

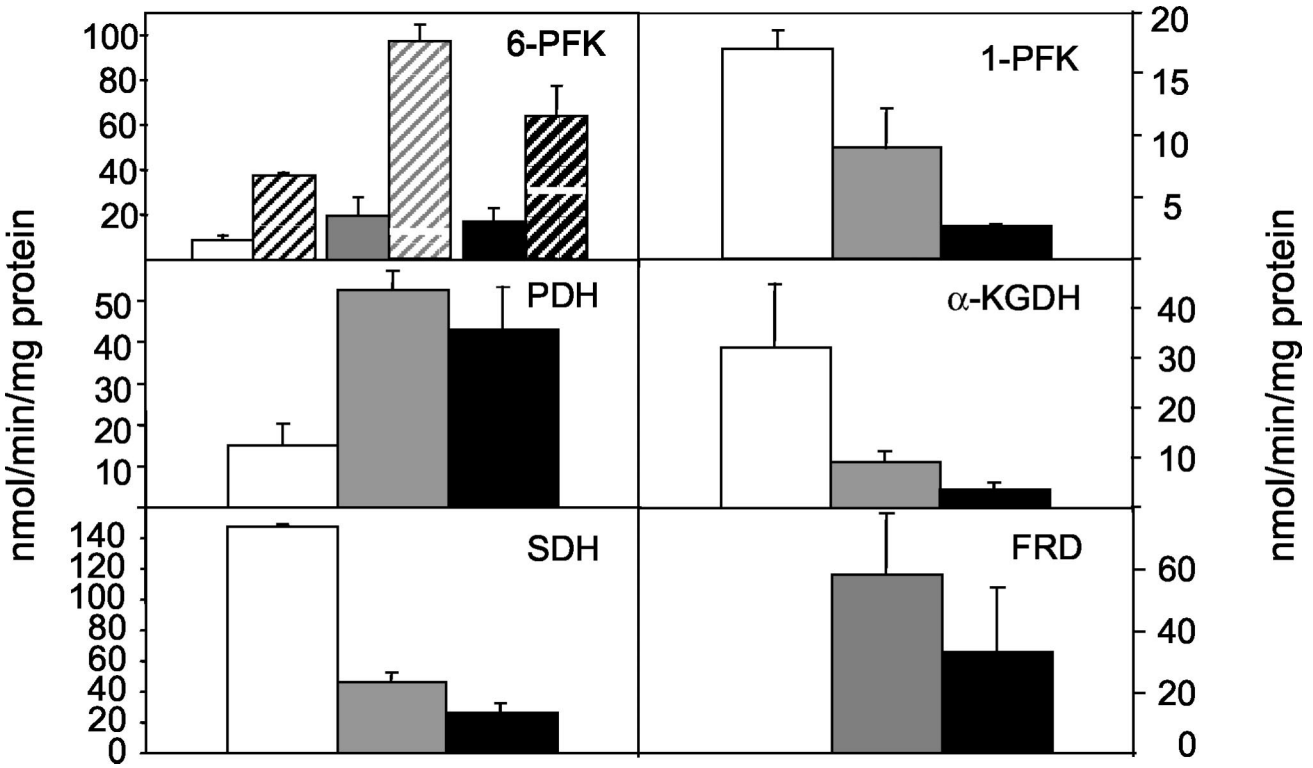

FIG. 2. In vitro activities of catabolic enzymes. Values represent the averages of three experiments; error bars represent maximal variations (percent) from the means. □, aerobic growth; ▨, microaerophilic growth; ■, anaerobic (phototrophic) growth. The ATP-dependent 6-PFK activities are also indicated as described above, whereas the PP_i-dependent enzymes are shown with hatched bars. The values under the white lines correspond to microaerophilic and anaerobic activities of PP_i-PFK in the absence of fructose (M medium).

obic, microaerophilic, and anaerobic (phototrophic) cells showed a trend similar to the results seen for the TH activities (presumably due to their colocalization in the PM), although the TH activity in microaerophilic cells was about twice that of aerobic cells. The relative amounts of TH and PM were correlated to the levels of *c*-type cytochromes, which were approximately the same for the microaerophilic and phototrophic (diminished-light) conditions. Under aerobic conditions, however, the level of BChl was almost negligible and *c*-type cytochromes were present at about 1 nmol/mg of protein (Fig. 3).

(iii) Activities of key TCA cycle enzymes in the microaerophilic state. In *E. coli* two key enzymes, αKGDH and SDH, have been shown to be important for the oxidative operation of the TCA cycle, because both enzymes are strongly repressed under anaerobic conditions, thus preventing the cycle from operating (2, 33). In *R. rubrum* the situation is different. Although aerobic cells possess high levels of αKGDH activity (about 32 nmol/min/mg of protein), anaerobic (phototrophic) cells still exhibit activity levels of about 10% of this value and the microaerophilic cells exhibit up to 29% of the levels obtained for aerobic cells grown in the dark (Fig. 2). These data support the very early results of Beatty and Gest (7, 8) for anaerobic cells of *R. capsulatus*, for which the αKGDH activity remained detectable. SDH was present at very high levels in aerobic cells (147 nmol/min/mg of protein), but significant activities were also detected in both phototrophic-anaerobic and microaerophilic cell extracts (46 nmol/min/mg of protein and 26 nmol/min/mg of protein, respectively) (Fig. 2). Interestingly, we were also able to detect significant FMNH-dependent FRD activities in microaerophilic extracts (Fig. 2) as well as in phototrophic extracts. The findings for FRD activities in extracts of phototrophically grown cultures are comparable with previous data (22). Surprisingly, the trends of the PDH profile did not correspond to those observed for SDH and αKGDH (Fig. 2). The highest levels of specific activity were observed for microaerophilic and anaerobic-phototrophic cell extracts (52 nmol/min/mg of protein and 43 nmol/min/mg of protein, respectively), with significantly lower levels of activity observed for aerobic cell extracts (15 nmol/min/mg of protein). We found no pyruvate-dependent reduction of methyl viologen, so the contribution of pyruvate-ferredoxin oxidoreductase to the measured PDH activity values is unlikely.

Rubisco activity was only found in phototrophic cells, presumably due to the well-known irreversible inactivation of this enzyme by molecular oxygen (13).

To test whether the lower levels of PM in microaerophilic cells were due solely to lower levels of ATP, we also grew *R. rubrum* microaerophilically in M2SF with dimethyl sulfoxide (DMSO) (0.5 M) as an electron acceptor. Although the reduction of DMSO by anaerobic respiration led to a higher cell yield than that seen with M2SF medium alone, the amount of PM (as indicated by the A_{880}/A_{660} ratio) produced per cell was only half of that observed in M2SF medium without DMSO (data not shown). In addition, it might be expected that under microaerophilic conditions, in which fermentative pathways play a significant role, PM production with growth on succinate

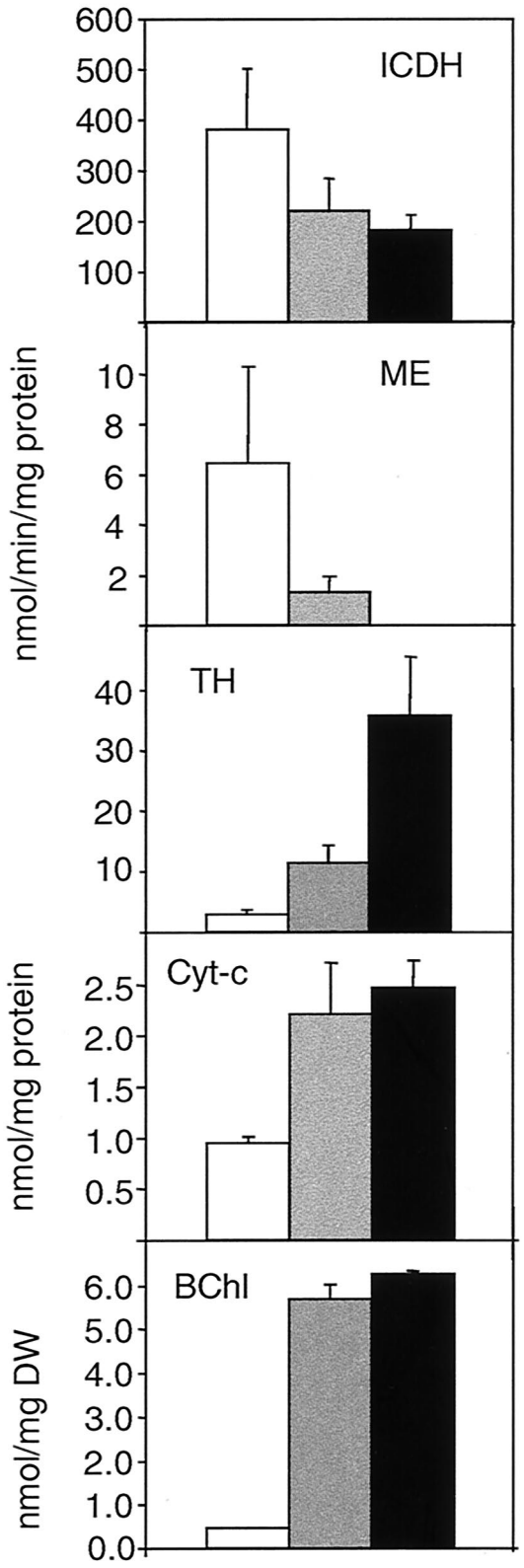

FIG. 3. In vitro activities of NADPH-generating enzymes and cellular contents of BChl and *c*-type cytochromes under different growth conditions. Values represent the averages of three experiments; error bars represent maximal variations (percent) from the means. □, aerobic growth; ▨, microaerophilic growth; ■, anaerobic (phototrophic) growth.

(which cannot be fermented) would be less than that with pyruvate (which can be fermented) (20). However, this was not the case; growth yields and levels of PM on both substrates were roughly equivalent (R. Ghosh, unpublished data).

DISCUSSION

In this study we defined the metabolic events involved in maintaining the microaerophilic growth condition commonly used for the induction of photosynthetic genes for the phenotypic characterization of photosynthetically incompetent mutants. The most striking and convenient indicator of this state is the production of BChl, which for *R. rubrum* is directly correlated to the expression and assembly of differentiated PM. Despite the widespread use of microaerophilic growth conditions for phenotype characterization, there is a paucity of information concerning the metabolic events required to maintain this state and which metabolic signals are involved in regulating gene expression.

In previous work (19), Ghosh et al. demonstrated that growth with succinate and fructose in the dark leads to maximal levels of PM under microaerophilic conditions. This succinate-fructose effect has so far only been reported for *R. rubrum* (although a similar effect has been observed for *Blastochloris viridis*, with the addition of glucose in place of fructose in the medium) (26) and presents a very useful tool for the elucidation of molecular signals which control PM induction.

Our enzymatic analysis of the microaerophilic cultures growing with succinate and fructose seems to indicate that the microaerophilic state requires a unique metabolic profile. In particular, significant levels of αKGDH and SDH were found under both microaerophilic and photoheterotrophic conditions (though at lower levels than those seen under aerobic conditions). These results indicate clearly that the oxidative TCA cycle plays a significant role in microaerophilic metabolism and are consistent with the previous observations that all TCA enzymes are present under either anaerobic (in the light) or aerobic (in the dark) conditions (3, 6). Anderson and Fuller (3) also showed that in photoheterotrophic cells grown on malate, the levels of αKGDH and SDH are very similar to those seen in cells grown autotrophically, although the level of citrate synthase activity was observed to be three- to fourfold lower. The significance of an operative oxidative TCA cycle becomes clear when coupled to the observation made here that the enzymes G6PDH and KDPG-aldolase could not be detected under any cultivation conditions. This contrasts with *R. capsulatus*, for which these enzymes have been reported to be present (12). The absence of a G6PDH activity or gene is consistent with the observations of Anderson and Fuller (3) that the PPP enzymes show almost no contribution to photoheterotrophic growth on malate and implies that this major source of NADPH generation for biosynthesis must be compensated by other pathways. Joshi and Tabita have shown that *R. rubrum* growing photoheterotrophically with malate possesses significant levels of NADP-dependent ICDH but that L-malic enzyme was absent (24). Under the conditions used here, the level of L-malic enzyme was also extremely low under microaerophilic conditions and ICDH levels were high. The observation of relatively high growth rates ($t_{1/2}$ = 7 h for microaerophilic growth), together with that of the low levels of

SDH and αKGDH (which limits the flux through the TCA cycle), leads us to suggest that the energy-driven TH reaction probably plays a major role in providing NADPH under microaerophilic and anaerobic growth conditions.

The high level of activity of PP_i-PFK revealed in this study shows clearly that PP_i-dependent enzymes are of considerable importance under all conditions, with microaerophilic cells exhibiting the highest activities. This is completely consistent with the fact that under microaerophilic conditions, the production of ATP is limited by the low level of oxidative phosphorylation and fermentative catabolism of fructose. We could find no kinase activity yielding fructose-6-phosphate in the cell extracts. This negative result supports the view that fructose is phosphorylated by the phosphotransferase system (with phosphoenolpyruvate as phosphate donor) and enters the cell solely as fructose-1-phosphate. In contrast to the ATP-dependent enzyme, PP_i-PFK catalyzes a fully reversible reaction (30). Thus, it is an attractive possibility that PP_i-PFK functions as a fructose-1,6-biphosphatase in the gluconeogenetic formation of glucose-6-phosphate under microaerophilic conditions, thereby generating PP_i, which contributes to the generation of proton motive force due to hydrolysis by membrane-bound inorganic pyrophosphatases. Furthermore, the proton potential generated by this reaction might couple PP_i metabolism to the TH reaction to enable the reduction of NADP under microaerophilic conditions in the dark.

The threefold-higher level of activity of PDH under microaerophilic conditions compared to aerobic conditions may be associated with its pivotal role in channeling carbon flux to acetyl-CoA under microaerophilic growth conditions, as was reported recently to be the case for the nonphotosynthetic bacterium *Azorhizobium caulinodans* (29). In that study aerobic acetyl-CoA formation was found to depend on reaction sequences of the oxidative TCA cycle (leading from oxaloacetate via malonate semialdehyde to acetyl-CoA), while under microaerophilic conditions (in which the TCA cycle is inhibited by the high [NADH]/[NAD] ratio), PDH proved to be essential for acetyl-CoA formation.

Tentative model of the microaerophilic state. For this study we employed the BChl and PM levels (indicated by the A_{880}/A_{660} ratio) of cultures (grown in the dark) as an indicator of the microaerophilic state. *R. rubrum* is experimentally advantageous in this respect, as these indicators are always correlated with the expression of the light-harvesting 1 complex, containing 95% of the BChl and associated reaction center. We note that to our knowledge and according to our own data, the degradation of BChl in *R. rubrum* is not significant. The apparent loss of BChl in the examined bioreactor cultures inoculated with an anaerobic light-grown culture was due simply to the O_2-induced arrest of PM biosynthesis, with subsequent dilution with increasing cell mass.

If one considers the production of PM to be a major phenotypic indicator of microaerophilic growth conditions, then two discrete states can be distinguished for *R. rubrum*. On one hand, growth in the presence of succinate alone leads to the production of PM at only low levels (20%) compared to those observed under phototrophic conditions (19). We define this level as PM_L. This microaerophilic state has been observed for nearly all facultative, purple nonsulfur bacteria investigated so far (16). On the other hand, when fructose is added to the growth medium as a second substrate, the PM level of *R. rubrum* increases to that of phototrophic cultures grown at low levels of light intensity. We designate this level PM_H.

The up-regulation of BChl due to the expression of light-harvesting 1 and reaction center complexes in response to decreased light intensity is probably mediated by redox control at the level of the quinone pool (28). This effect is qualitatively and quantitatively reminiscent of the up-regulation of these complexes induced by the addition of fructose as a second substrate in the microaerophilic state. We suggest here that in *R. rubrum*, these regulatory processes are fundamentally related at the molecular level and are primarily regulated by the metabolic profiles observed in the presence and absence of fructose under different growth conditions.

A fundamental tenet of our model is that only the [NADH]/[NAD] and $[UQH_2]/[UQ]$ ratios (and not the [NADPH]/[NADP] ratios) influence the signal transduction components which respond to changes in light intensity via the quinone pool to increase the levels of PM. We propose that in *R. rubrum* cells growing microaerophilically, the PM_L and PM_H states correspond to two discernible physiological redox states [designated here $(M\text{-}AER)_{OX}$ (microaerophilic oxidative mode) and $(M\text{-}AER)_R$ (microaerophilic reductive mode)] which are determined by the balance of carbon flux and the production and utilization of reducing equivalents. The results obtained using the DO-pH control loop as described above indicate clearly that the carbon flux and production and utilization of reducing equivalents at the level of the TCA cycle appear to be delicately poised between oxidative and reductive modes of metabolism.

In the $(M\text{-}AER)_{OX}$ state, the [NADH]/[NAD] ratio is low due to the depletion of NADH by both electron flow to O_2 and the TH reaction. As a consequence, the $[UQH_2]/[UQ]$ ratio is low and PM expression is weak (PM_L). If additional redox equivalents arising from fructose metabolism are provided simultaneously, the [NADH]/[NAD] ratio (and thus the $[UQH_2]/[UQ]$ ratio) remains high and is sufficient to signal increased levels of PM (PM_H). In this state, the TCA cycle is hovering between reductive (FRD-driven) and oxidative (SDH-driven) modes. This is the $(M\text{-}AER)_R$ state.

Interpretation of the experimental data for the microaerophilic state with the two-state model. The above-described model enables the interpretation of the carbon fluxes observed in the DO-pH control experiment described in Materials and Methods. In the initial growth phase in the presence of fructose and under conditions in which the pO_2 was maintained at 5% (Fig. 1A), succinate and fructose are catabolized principally via the EMP pathway and the oxidative TCA cycle. After the transition to microaerophilic conditions is initiated, the pO_2 level drops to less than 0.3%; the breakdown of fructose via PP_i-PFK then dominates over succinate consumption, because the αKGDH activity is largely repressed. This condition forces the TCA pathway to function partially reductively, as indicated by the appearance of propionate in the medium (data not shown), but a small amount of αKGDH is maintained to allow low-level oxidative metabolism. As a corroboration of these data, we have detected considerable amounts of succinate (in both phototrophically grown cultures and cultures grown fermentatively in the dark in which only fructose was used as a carbon source) as well as significant levels of

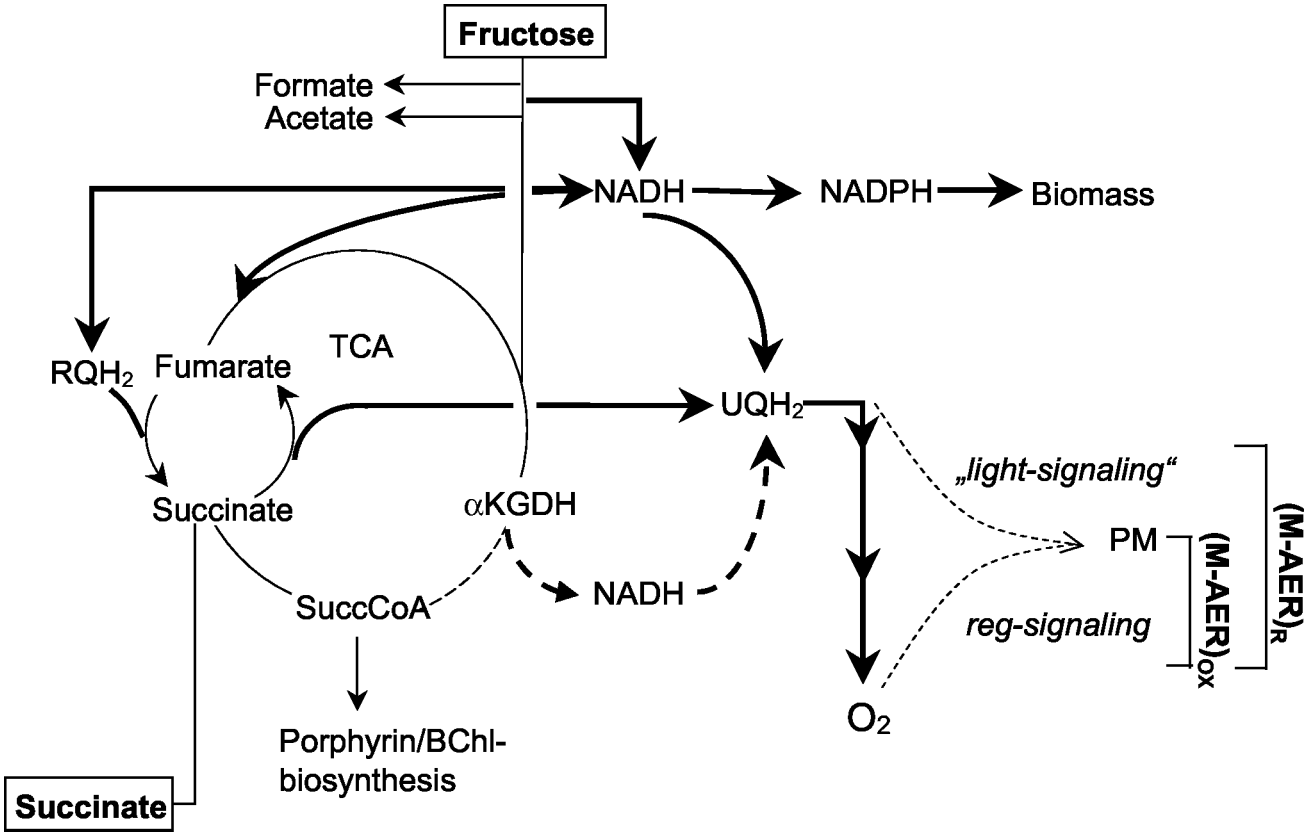

FIG. 4. Major flow of carbon (thin lines) and reducing equivalents (thick lines) and redox (*reg*) signaling during microaerophilic growth in M2SF medium. RQH_2, rhodoquinone (reduced).

FRD under microaerophilic and anaerobic phototrophic conditions. We note in passing that in *R. rubrum*, FRD requires a high-potential benzoquinone, rhodoquinone ($E_0' = -63$ mV), instead of ubiquinone ($E_0' = +70$ mV) as a substrate for fumarate reduction (22, 31) (Fig. 4).

The increased carbon flux from fructose breakdown causes a buildup of acetate via acetyl-kinase. The low levels of formate found in the medium after 17 h suggest that the contribution of pyruvate-formate lyase is minor. The redox equivalents arising from the oxidation of fructose via the EMP pathway, the PDH reaction, and the TCA cycle yielded sufficient NADH to maintain *R. rubrum* in the $(M\text{-}AER)_R$ state. At 40 h, fructose had been consumed completely and the $(M\text{-}AER)_{OX}$ state was generated due to the depletion of reducing equivalents; this caused a repression of PM production (Fig. 1A), and succinate as well as acetate were now utilized as substrates for growth, probably via the citramalate bypass of the TCA cycle and involving citramalyl-CoA lyase and malate synthase, as recently described (23).

In the pO_2-controlled experiments, the pO_2 was initially high but decreased steeply during the initial 20 h. Under these conditions, the TCA pathway functioned exclusively as an oxidative cycle. NADPH was provided by ICDH, malic enzyme, and the proton motive force (Δp)-dependent TH. After 20 h the pO_2 was maintained at about 0.5%, which was sufficient to still drive considerable flux through the oxidative TCA pathway, allowing the simultaneous consumption of both fructose and succinate. NADH was depleted by the Δp-dependent TH reaction as well as low-level aerobic respiration. Since PM biosynthesis was arrested under these conditions (Fig. 1B), we understand the cells to have been in the $(M\text{-}AER)_{OX}$ state, presumably due to the large pO_2 fluctuations associated with the pO_2 electrode control at levels below 1% pO_2.

Our metabolic model for the microaerophilic state proposes that the transition from the $(M\text{-}AER)_{OX}$ state to the $(M\text{-}AER)_R$ state is mediated simply by the level of $[UQH_2/UQ]$, which is dependent upon the presence of the NADH pool. The involvement of $[UQH_2/UQ]$ is consistent with models for the regulation of gene expression as described by Oh and Kaplan (28) and Bauer and coworkers (6). In these models, O_2 regulation is mediated by the *prr* (17) and *reg* (34) systems but further regulation ("light signaling") is mediated by the UQ pool via a number of signal transduction components. Our model adds a further layer to the existing models by allowing for rapid transitions between reductive and oxidative pathways in response to the pO_2 level. The model predicts that modifications which affect either UQH_2- or NADH-generating pathways or the relative carbon flux through the TCA pathway should have significant effects upon the level of PM expression. Future work will examine these predictions both experimentally and theoretically.

ACKNOWLEDGMENTS

This work was supported by the "Landesschwerpunkt Biosystemtechnik" from the University of Stuttgart and the State Baden-Württemberg, Germany.

We thank Khaled Abou-Aisha and Henrik Sommer for their help with gene annotations.

REFERENCES

1. **Ackrell, B. A. C., E. B. Kearny, and T. P. Singer.** 1978. Mammalian succinate dehydrogenase. Methods Enzymol. **53:**466–483.
2. **Amarasingham, C. R., and B. D. Davis.** 1965. Regulation of α-ketoglutarate dehydrogenase formation in *Escherichia coli.* J. Biol. Chem. **240:**3664–3668.
3. **Anderson, L., and R. C. Fuller.** 1967. Photosynthesis in *Rhodospirillum rubrum.* III. Metabolic control of reductive pentose phosphate and tricarboxylic acid cycle enzymes. Plant Physiol. **42:**497–502.
4. **Baltscheffsky, H. L., V. Von Stedingk, H. W. Heldt, and M. Klingenberg.** 1966. Inorganic pyrophosphate: formation in bacterial photophosphorylation. Science **153:**1120–1122.
5. **Baltscheffsky, M., S. Nadanaciva, and A. Schultz.** 1998. A pyrophosphate synthase gene: molecular cloning and sequencing of the cDNA encoding the inorganic pyrophosphate synthase from *Rhodospirillum rubrum.* Biochim. Biophys. Acta **1364:**301–306.
6. **Bauer, C., S. Elsen, L. R. Swem, D. L. Swem, and Shinji Masuda.** 2003. Redox and light regulation of gene expression in photosynthetic prokaryotes. Philos. Trans. R. Soc. Lond. B Biol. Sci. **358:**147–154.
7. **Beatty, J. T., and H. Gest.** 1981. Biosynthetic and bioenergetic functions of citric acid cycle reactions in *Rhodopseudomonas capsulata.* J. Bacteriol. **148:** 584–593.
8. **Beatty, J. T., and H. Gest.** 1981. Generation of succinyl-coenzyme A in photosynthetic bacteria. Arch. Microbiol. **129:**335–340.
9. **Bergmeyer, H. U.** 1995. Methods of enzymatic analysis, vol. 3. VCH, Weinheim, Deerfield Beach, Fla.
10. **Bonora, P., I. Principi, A. Hochkoeppler, R. Borghese, and D. Zannoni.** 1998. The respiratory chain of the halophilic anoxygenic purple bacterium *Rhodospirillum sodomense.* Arch. Microbiol. **170:**435–441.
11. **Clayton, R. K.** 1963. Towards the isolation of a photochemical reaction center in *Rhodopseudomonas sphaeroides*. Biochim. Biophys. Acta **7:**312–323.
12. **Conrad, R., and H. G. Schlegel.** 1977. Different degradation routes for glucose and fructose in *Rhodopseudomonas capsulata.* Arch. Microbiol. **112:** 39–48.
13. **Cook, L. S., and F. R. Tabita.** 1988. Oxygen regulation of ribulose 1,5-bisphosphate carboxylase activity in *Rhodospirillum rubrum.* J. Bacteriol. **170:**5468–5472.
14. **Cotton, P. J., T. M. Lever, B. F. Nore, M. R. Jones, and J. B. Jackson.** 1989. The coupling between protonmotive force and the $NAD(P)^+$ transhydrogenase in chromatophores from photosynthetic bacteria. Eur. J. Biochem. **182:**593–603.
15. **Daniels, G. A., G. Drews, and M. H. Saier, Jr.** 1988. Properties of a Tn*5* insertion mutant in the structural gene (*fruA*) of the fructose-specific phosphotransferase system of *Rhodobacter capsulatus* and cloning of the *fru* regulon. J. Bacteriol. **170:**1698–1703.
16. **Drews, G., and J. R. Golecki.** 1995. Structure, molecular organization, and biosynthesis of membranes of purple bacteria, p. 231–257. *In* R. E. Blankenship, M. T. Madigan, and C. R. Bauer (ed.), Anoxygenic photosynthetic bacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands.
17. **Eraso, J. M., and S. Kaplan.** 1994. PrrA, a putative response regulator involved in oxygen regulation of photosynthesis gene expression in *Rhodobacter capsulatus.* J. Bacteriol. **176:**32–43.
18. **Forward, J. A., M. C. Behrendt, N. R. Wyborn, R. Cross, and D. J. Kelly.** 1997. TRAP transporters: a new family of periplasmic solute transport systems encoded by the *dctPQM* genes of *Rhodobacter capsulatus* and by homologs in diverse gram-negative bacteria. J. Bacteriol. **179:**5482–5493.
19. **Ghosh, R., A. Hardmeyer, I. Thoenen, and R. Bachofen.** 1994. Optimization of the Sistrom medium for large-scale batch cultivation of *Rhodospirillum rubrum* under semiaerobic conditions with maximal yield of photosynthetic membranes. Appl. Environ. Microbiol. **60:**1698–1700.
20. **Gorrel, T. E., and R. L. Uffen.** 1977. Fermentative metabolism of pyruvate by *Rhodospirillum rubrum* after anaerobic growth in the dark. J. Bacteriol. **131:**533–543.
21. **Govantes, F., A. V. Orjalo, and R. P. Gunsalus.** 2000. Interplay between three global regulatory proteins mediates oxygen regulation of the *Escherichia coli* cytochrome *d* oxidase (*cydAB*) operon. Mol. Microbiol. **385:**1061–1073.
22. **Hiraishi, A.** 1988. Fumarate reduction systems in members of the family *Rhodospirillaceae* with different quinone types. Arch. Microbiol. **150:**56–60.
23. **Ivanovskii, R. N., E. N. Krasilnikova, and I. A. Berg.** 1997. The mechanism of acetate assimilation in the purple nonsulfur bacterium *Rhodospirillum rubrum* lacking isocitrate lyase. Microbiology **66:**744–749.
24. **Joshi, H. M., and F. R. Tabita.** 2000. Induction of a carbon monoxide dehydrogenase to facilitate redox balancing in a ribulose bisphosphate carboxylase/oxygenase-deficient mutant strain of *Rhodospirillum rubrum.* Arch. Microbiol. **173:**193–199.
25. **Kersters, K., and J. De Ley.** 1971. Enzymatic tests with resting cells and cell-free extracts. Methods Microbiol. **6**:3–52.
26. **Lang, F. S., and D. Oesterhelt.** 1989. Microaerophilic growth and induction of the photosynthetic reaction center in *Rhodopseudomonas viridis.* J. Bacteriol. **171:**2827–2834.
27. **Law, J. H., and R. A. Slepecky.** 1961. Assay of poly-hydroxybutyric acid. J. Bacteriol. **82:**33–36.
28. **Oh, J. I., and S. Kaplan.** 2001. Generalized approach to the regulation and integration of gene expression. Mol. Microbiol. **39:**1116–1123.
29. **Pauling, D. C., J. P. Lapointe, C. M. Paris, and R. A. Ludwig.** 2001. *Azorhizobium caulinodans* pyruvate dehydrogenase activity is dispensable for aerobic but required for microaerophilic growth. Microbiology **147:**2233–2245.
30. **Pfleiderer, C., and J. H. Klemme.** 1980. Pyrophosphat-abhängige D-fructose-6-phosphat phosphotransferase in *Rhodospirillaceae*. Z. Naturforsch. Sect. C **35**:229–238.
31. **Ramirez-Ponce, M. P., J. M. Ramirez, and G. Gimenez-Gallego.** 1980. Rhodoquinone as a constituent of the dark electron-transfer system of *Rhodospirillum rubrum.* FEBS Lett. **119:**137–140.
32. **Sistrom, W. R.** 1960. A requirement for sodium in the growth of *Rhodopseudomonas sphaeroides.* J. Gen. Microbiol. **22:**778–785.
33. **Spencer, M. E., and J. R. Guest.** 1987. Regulation of citric acid cycle genes in facultative bacteria. Microbiol. Sci. **4:**164–168.
34. **Swem, L. R., S. Elsen, T. H. Bird, D. L. Swem, H.-G. Koch, H. Myllykallio, F. Daldal, and C. E. Bauer.** 2001. The RegB/RegA two-component regulatory system controls synthesis of photosynthesis and respiratory electron transfer components in *Rhodobacter capsulatus.* J. Mol. Biol. **309:**121–138.
35. **Tseng, C. P., J. Albrecht, and R. P. Gunsalus.** 1996. Effect of microaerophilic cell growth conditions on expression of the aerobic (*cyoABCDE* and *cydAB*) and anaerobic (*narGHJI*, *frdABCD*, and *dmsABC*) respiratory pathway genes in *Escherichia coli.* J. Bacteriol. **178:**1094–1098.